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MORPHOLOGICAL OBSERVATIONS AND MICROSPECTROPHOTOMETRIC DATA FROM PHOTORECEPTORS IN THE RETINA OF THE SEA RAVEN, *HEMITRIPTERUS AMERICANUS*

B. A. COLLINS* AND E. F. MACNICHOL, JR.*#

**Laboratory of Sensory Physiology, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, and #Department of Physiology, Boston University School of Medicine, Boston, Massachusetts 02118*

Abstract

A histological survey of the retinas of some fishes has revealed an unusual cone formation. We repeatedly find these unusual triple cones in 10% of the retinas reviewed. To obtain further information about these photoreceptors, the sea raven, whose retina is known to contain them, was chosen for further study. Microspectrophotometric measurements were made to determine the peak absorbance of the visual pigment contained in the three individual outer segments. We report here the results of measurements made on triple cones found in the retina of the sea raven, *Hemitripterus americanus*.

Introduction

In a continuing histological survey of the retinas of fishes indigenous to the Woods Hole, Massachusetts area, numerous preparations are reviewed (Collins and MacNichol, 1978). An unusual cone type, a triple cone (Vrabec, 1955; Ali and Anctil, 1976) appears in approximately 10% of the fish retinas prepared (Collins and MacNichol, 1979). Its development and function is not known. Most of the fish species studied have two morphologically different cone types, double cones and single cones. Microspectrophotometric data on these cone types indicates that the paired outer segments of the double cones usually contain either two long-wave ("red-red") or two middle-wave ("green-green") visual pigments; or one of the outer segments contains the long-wave, the other the middle-wave pigment ("red-green"). The outer segment of the smaller single cone usually bears the short-wave ("blue") visual pigment (Stell and Hárosi, 1976; Levine and MacNichol, 1979; Hárosi, 1982).

Triple cones are approximately the same size as double cones and, indeed, can be easily overlooked. We first saw these triple cones in histological preparations which were made by sectioning tangentially at the ellipsoid level of the retina. Once it became apparent that this unusual cone formation was not artifactual, it was decided to obtain further information about these interesting photoreceptors.

Since the histological preparations were made from randomly chosen tissue throughout the retina of the sea raven, it was not known where the triple cones would be located. Also, having made a great number of fresh and partially fixed (Levine *et al.*, 1981) retinal preparations from many of these fish, it appeared that these unusual cones were not in every individual studied. Therefore, we considered these photoreceptors quite rare.

Hárosi (1982) reported preliminary microspectrophotometric (MSP) data on the visual pigments from one triple cone found in the retina of the sea raven, *Hemitripterus*

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americanus. All three outer segments of this triad contained the same green-sensitive pigment. His MSP results and our earlier data, showed that the double cones of the sea raven can either bear the same ("green-green") or different ("red-green") visual pigments. The present investigation was undertaken in an attempt to determine, if indeed, some of the triple cones contain different visual pigments in their outer segments.

Materials and Methods

Sea raven were collected from the waters of the Woods Hole area by research vessels of the National Marine Fisheries Service, Northeast Fisheries Center or by Marine Biological Laboratory vessels. Since sea raven prefer cool, deep waters (Bigelow and Schroeder, 1953), they were best caught in the late fall and throughout the winter season. Many died when they were hauled up from the deep, and those that survived died in our running sea water tanks if the water temperature rose above 55°F. All fish used in this study were more than 8 inches in body length, with some over 15 inches long. No attempt was made to determine their age or sex.

Each sea raven was dark-adapted for at least two hours in oxygenated sea water in a light-tight tank, then anesthetized with tricaine methanesulfonate. Under infrared light the eye was enucleated and hemisected, then the eye cup was placed in a teleost Ringer's solution with the following composition: NaCl (133.5 *M*m), KCl (3.4 *M*m), $MgCl_2$ (9.8 *M*m), NaH_2PO_4 (2.9 *M*m), $NaHCO_3$ (11.9 *M*m), $CaCl_2$ (1.5 *M*m), glucose (11.1 *M*m) with the pH adjusted to 7.8 (this yields approximately 330 mOs/kg). The pigment epithelium separates readily from the sensory retina in 15–30 minutes after immersion in the Ringer's solution. A small piece (*ca.* 1 mm^2) of retinal tissue was cut and, using a pipette, was transferred in a drop of the Ringer's solution to a coverslip. The tissue was surrounded by a ring of silicone oil (Dow Corning #702), and another coverslip was laid carefully on top of the tissue (MacNichol *et al.,* 1978). This coverslip sandwich was transferred to the microscope of the photon-counting microspectrophotometer (PMSP) (MacNichol, 1978). With the aid of the infrared video monitor attached to the PMSP, the preparation was scanned to locate the various cone types. Once a triple cone was located, transverse measurements were made through the outer segments and the visual pigment spectrum was recorded (Stell and Hárosi, 1976; Levine and MacNichol, 1979; Hárosi, 1982; MacNichol *et al.,* 1983). After these measurements were made, the preparation was transferred to a Nomarski differential interference-contrast microscope and an attempt was made to locate and photograph the same triple cone with a Polaroid camera attached to the microscope.

Results

Histological observations of tangentially sectioned preparations through the ellipsoid region of the retina of the sea raven show that photoreceptors form a regular mosaic pattern. As found in many teleosts, four double cones form a square with a single cone in the center (Ali and Anctil, 1976; Kunz, 1980) (Fig. 1a). However, occasionally a triple cone would appear randomly placed within this mosaic pattern (Fig. 1b, c).

In sea raven the two members of the double cones are equal in size, having inner segments approximately 22–25 μm long and 12–15 μm wide, with outer segments about 15 μm long by 3–5 μm wide. Because the three members of the triple cones

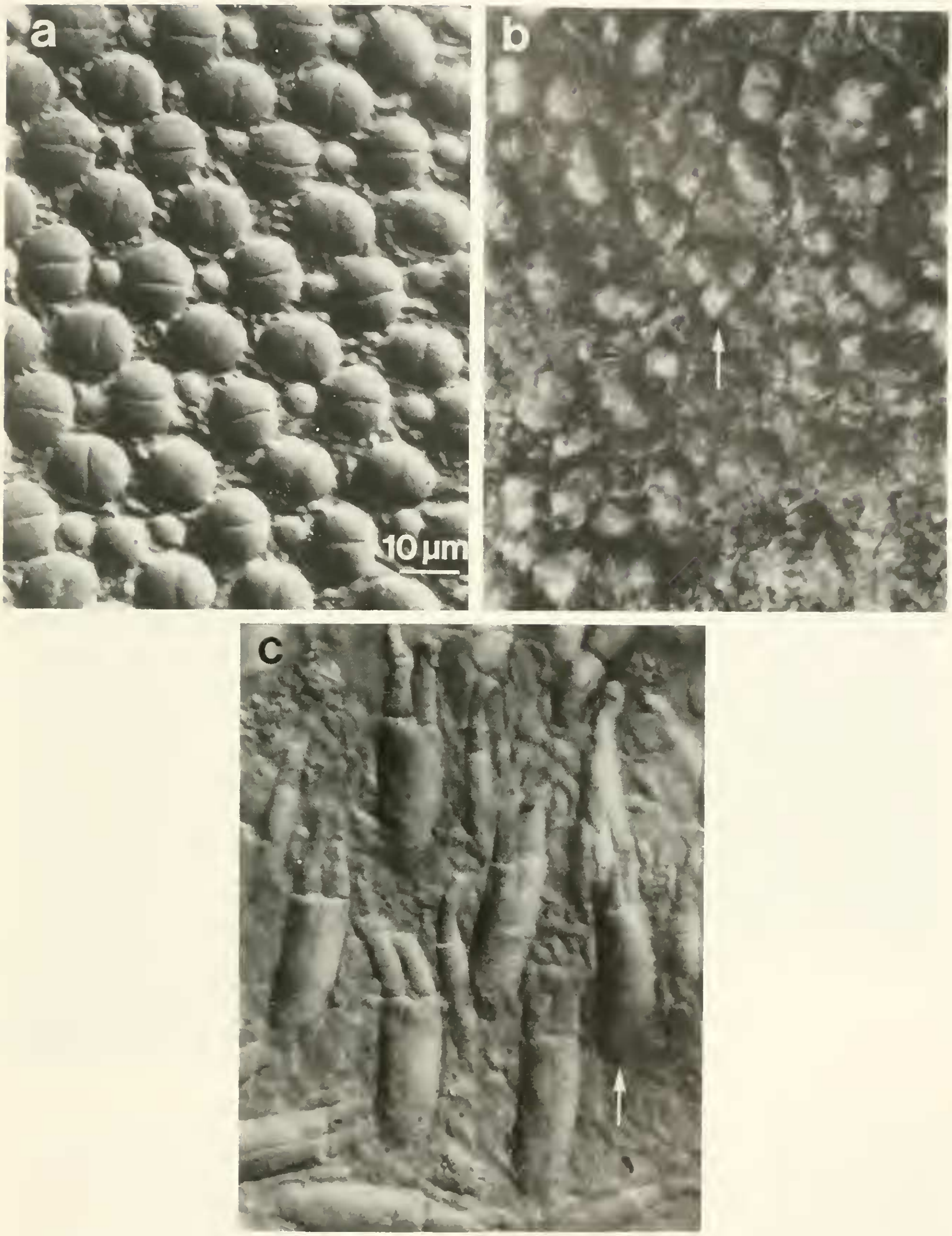

FIGURE 1. Mosaic patterns of the retina of the sea raven. (a) A photomicrograph of a histological preparation of sea raven retina. The preparation was made by sectioning tangentially through the ellipsoid region. A square mosaic pattern is formed by the double and single cones. The photograph was taken using a Polaroid camera attached to a Nomarski interference-contrast microscope. The microscope objective was 40×, the camera eyepiece was 12.5×. (b) Photomicrograph of a fresh whole-mount preparation from the sea raven retina. A view at the ellipsoid level which shows a triple cone appearing among the double and single cones. Arrow points to triple cone. Magnification is the same as in Figure 1a. (c) A photomicrograph of a partially fixed retinal preparation showing double cones that form the square pattern around the single cones of the sea raven. An arrow points to the triple cone that can be seen randomly placed in the mosaic pattern. Magnification is the same as in Figure 1a.

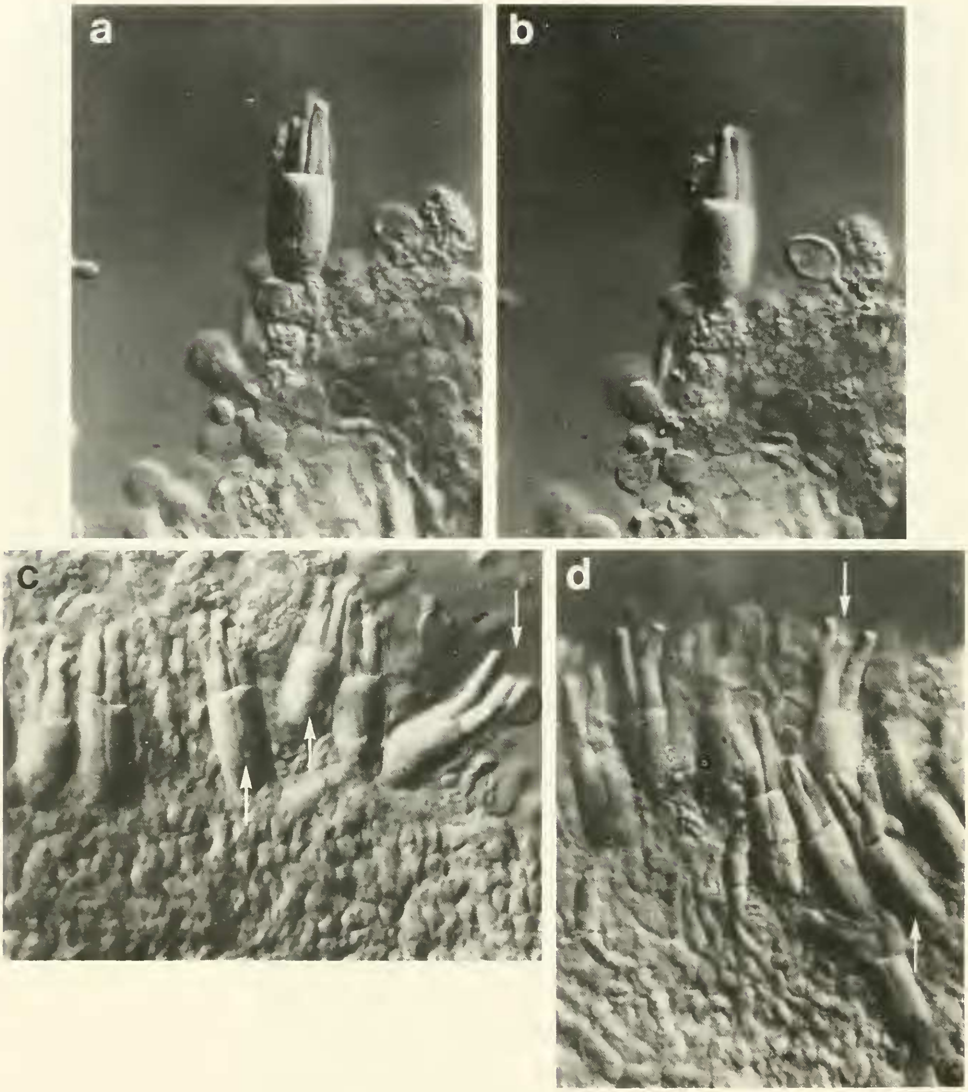

FIGURE 2. Triple cones from the retina of the sea raven. (a) Photomicrograph of an isolated triple cone from partially fixed retinal tissue of the sea raven. The magnification is the same as in Figure 1a. (b) Photomicrograph of the same cone as in Figure 2a. Microscope focus changed slightly bringing the foreground area, and the third member of the triple cone, into focus. Same magnification as Figure 1a. (c) (d) Photomicrographs of clusters of triple cones, interspaced among double and single cones, found in a partially fixed piece of retinal tissue from the sea raven. Arrows point to triple cones. Magnification as in Figure 1a.

are so similar, and most often two of the three outer segments are over-lying, they could easily be mistaken for double cones (Fig. 2a, b). However, once a triple cone was located, several more would often be seen in the vicinity (Fig. 2c, d). The inner segment of the single cone is approximately 12 μm long and 6 μm wide. The outer segment of the single cone is approximately 15 μm long and 3–5 μm wide, corresponding in size to the double cone.

The sea raven has large photoreceptors, and the comparative ease of enucleation and dissection of the retina makes it an ideal model for numerous visual studies. During various seasons over a period of several years, many sea ravens have been used in our laboratory. Whenever PMSP measurements were made on the retina of this fish, we looked for triple cones (Fig. 3a, b). During this time the outer segments of sixteen triple cones were measured from five different individuals although this cone type was seen in preparations made from the many sea raven retinas reviewed. If the patch of retinal tissue being examined by PMSP contained triple cones, usually only one or two were free from surrounding tissue. Too often, none of the outer segments of the triple cones were optically isolated enough to be measured. From one fish, however, a piece of the retinal tissue yielded seven measurable triples. Occasionally PMSP recordings were made through two over-lying outer segments; this increased the optical density, but the wavelength of peak absorbance (λ_{max}) and half band-width was the same as measurements taken from a single, isolated outer segment of a triple cone if the outer segments bore the same visual pigments. Pigment measurements were accepted according to the criteria of MacNichol *et al.* (1983).

When obtaining PMSP measurements from the double cones, it was noted that the percentage of "green-green" pairs was considerably higher than the "red-green" absorbing pair of outer segments of these cones. From a total of 70 acceptable records, the λ_{max} of 58 green-absorbing cone outer-segments was 521.7 ± 3.28 nm (S.D.) and the λ_{max} of the 12 red-absorbing cone outer-segments was 560.1 ± 5.25 nm (S.D.). All 22 single cones measured contained a blue-absorbing pigment with a λ_{max} at 462.5 ± 3.72 nm (S.D.); the λ_{max} of the pigment in the numerous rods was 505 ± 2.87 (S.D.). These results are in close agreement with the findings of Hárosi (1982). In fourteen triple cones, all three outer segments contained the same green-sensitive visual pigment (Fig. 3c). Only three of these triple cones yielded data that were acceptable; but from these, the λ_{max} was determined to be 522.1 ± 5.56 nm (S.D.). However the outer segments of two triple cones contained different visual pigments. PMSP results indicated that two of the three outer segments were green-absorbing and the third bore the red-absorbing pigment. From one of these cones, PMSP scans were taken through the three individual outer segments (Fig. 3d). The better record was made of the second triple cone (Fig. 3e) whose two green-sensitive outer segments were overlapping, yielding an optical density of 0.112 OD which was nearly double that of the third, red-sensitive member (0.061 OD).

Discussion

Histological studies of the retina indicate that sea raven possess a well developed visual system; photopigment data suggests that this fish has the physical capacity to discriminate colors. Such a complex visual system might be unnecessary if this fish remained in the dim narrow-band illumination of the deep water for its entire life cycle. The sea raven is normally found down to 50 fathoms, but it has been taken as deep as 105 fathoms (Bigelow and Schroeder, 1953). Deep water transmits only a narrow blue-green band of the visible spectrum. Receptors having sensitivities outside this range might be of little use, unless they are used to observe bioluminescence which is not present in the raven's normal habitat.

For most of the year adult sea ravens vary in color from reddish purple to chocolate or gray with a yellowish belly. During the breeding season they move to shallow

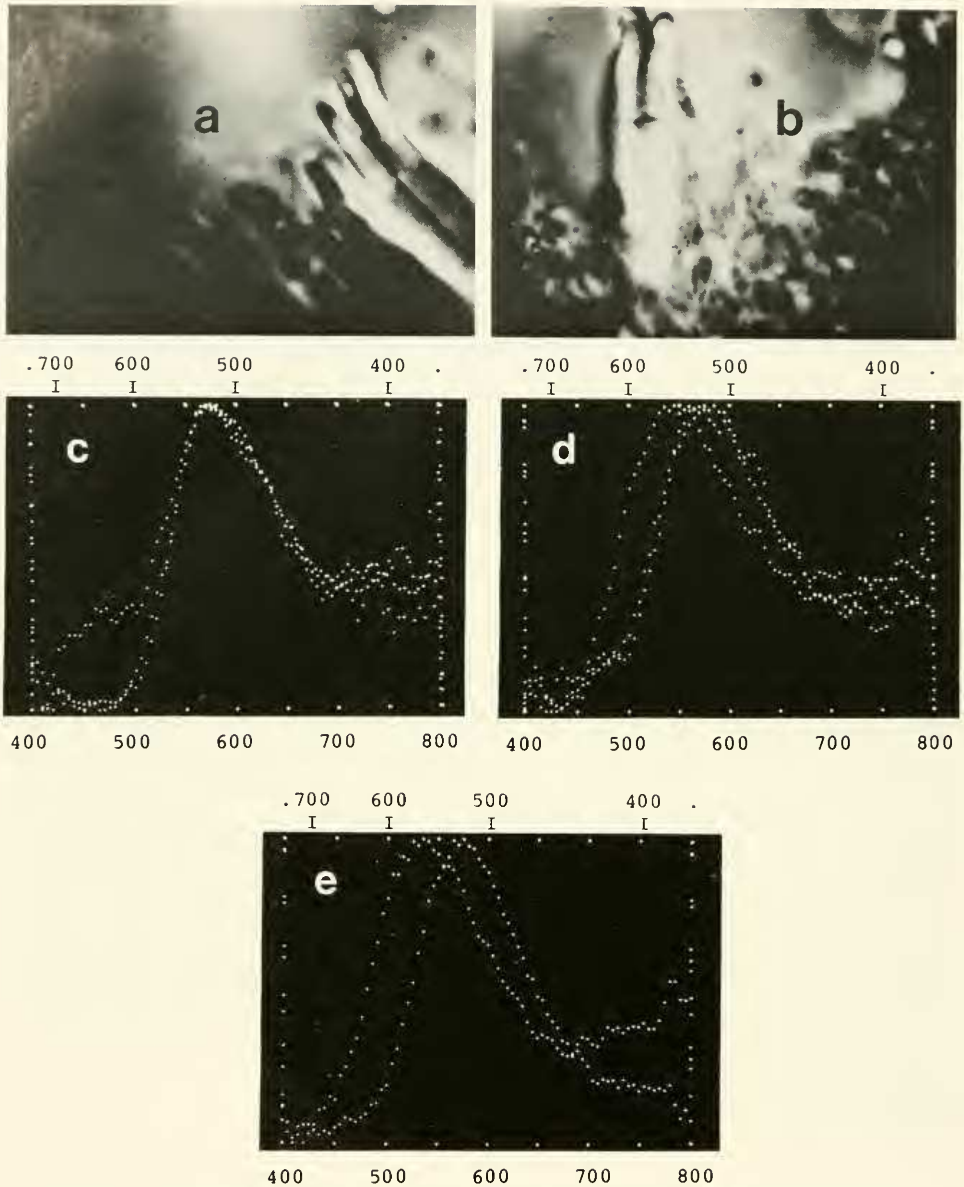

FIGURE 3. Triple cones photographed from the infrared video monitor of the PMSP at the time of measurement, and the records of some of the measurements obtained. (a) Note the white spot in the center member of the three outer segments illustrated in this photograph; this is the measuring beam of the PMSP. The magnification is slightly higher than in Figure 1a. (b) The three outer segments of the triple cone are tightly clumped together making individual PMSP measurements difficult to obtain. The magnification is the same as in Figure 3a. (c) Superimposed records of the pigment absorbance spectra from all three outer segments of a triple cone found in the retina of the sea raven. This record indicates that each outer segment bears the same green-absorbing pigment. The measured values of λ_{max} are: 519, 519, and 523 nm. (d) Superimposed records of the pigment absorbance spectra from the three outer segments of another triple cone showing that one of the members is red-absorbing while the other two are green-absorbing. (Measured λ_{max} = 554, 524, and 524 nm.) (e) Superimposed records from a second triple cone bearing one red-absorbing member. One scan was made through the red-absorbing outer segment. The other was made with the beam passing in succession through both green-absorbing outer segments since they were overlapping; this yielded nearly double the optical density of the first member. (Measured λ_{max} = 565 and 525 nm.) All records are displayed on a linear frequency scale indicated in Terahertz by the numbers at the bottom. The upper non-linear scale is in nanometers. (Thz = 100,000/nm = 10^{12} Hz). The individual points are at 5 THz intervals and are derived from total photon counts in the 5 THz region surrounding each point.

water where a broad spectrum of illumination is available, and assume a brillant body coloration, from bright yellow through orange to blood-red (Goode, 1888). In southern New England, from early October to late December, the sticky egg clusters of the sea raven are deposited on the branches of the pinkish finger sponge, *Chalina*, or on the yellow breadcrumb sponge, *Halichondria* (Warfel and Merriman, 1944). A highly developed ability to make visual discriminations at this critical part of the life cycle could be an important advantage in recognition of both a suitable mate and suitable area for egg deposition.

McFarland and Munz (1975) measured the characteristics of the photic environment of the surface, middle, and bottom dwelling fishes. Microspectrophotometric measurements of the visual pigments of some of these fishes suggest that color sensitivity evolved by a fish species may be related to the photic environment in which it lives (Levine and MacNichol, 1979). For example, surface dwelling fishes are sensitive throughout the visible spectrum, indeed some have photoreceptors sensitive into the ultraviolet (Hárosi and Hashimoto, 1983), whereas the sensitivity of those fishes dwelling near the bottom is greatest in the blue-green region of the spectrum.

The behavior of the sea raven indicates that it ranges over a variety of habitats with varying photic environments. The microspectrophotometric measurements of the photopigments of this fish suggests that its visual system is capable of coping with these environmental changes. The sea raven lives in deep water for most of the year. At this time perhaps the triple cone, increasing the visual sensitivity in the blue-green region, becomes extremely important for the survival of this species. Whereas during the three month breeding season, the long-wavelength region of the raven's visual system is utilized for the all important reproduction cycle and its egg deposition.

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